Environmental Science and Engineering

Shyama Prasad Saha
Deepika Mazumdar
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Piyush Mathur *Editors*

Agro-waste to Microbe Assisted Value Added Product: Challenges and Future Prospects

Recent Developments in Agro-waste Valorization Research

Springer

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Preface

Agro-waste valorization refers to the process of converting agricultural waste into valuable products through the use of microbes and microbial biotechnology. This book will comprehensively describe the latest research and technologies that harness the power of microbial biotechnology to convert agro-waste into economically valuable products. The major part of the book has stressed upon the application of various bacteria and fungi for agro-waste valorization and their ability to carry out these transformations under mild conditions, reducing energy requirements compared to traditional chemical processes. An important section of the book will also focus on the utilization of different forms of agro-waste in a sustainable manner and their transformation into various commercial by products such as biofuels, bioplastics, enzymes, organic fertilizers, and other high-value compounds. The book will also unlock the advantages of microbial biotechnology for sustainably utilizing renewable resources and explore its significant role in the enzyme industry and nanobiotechnology. The book will also deliver information on different mechanisms of agro-waste valorization by bacteria and fungi, that play a crucial role in breaking down organic matter present in agro-waste and converting it into useful substances. Undoubtedly, the book will prove to be a comprehensive guide, designed to empower researchers, scientists, and enthusiasts working in the field of industrial microbiology, microbial biotechnology, agricultural biotechnology, etc. Additionally, the book will unleash the power of microbes for a greener and more efficient future as well as agro-waste management.

Siliguri, India

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About the Editors

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Dr. Deepika Mazumdar is currently working as an Assistant Professor in the Department of Microbiology, North Bengal St. Xavier's College, Jalpaiguri. She has been teaching for the fast 5 years. She has extensive research experience of more than 11 years and completed her Ph.D. from the Department of Biotechnology, University of North Bengal. In her Ph.D. journey, she worked with Plant Growth-Promoting Rhizobacteria (PGPR) and studied their effect on plant growth promotion. She had good number of research publications in various international high-impact peer-reviewed journals. She participated and presented her work at various national and international conferences.

Dr. Swarnendu Roy works as Assistant Professor at the University of North Bengal, Siliguri, India. He has more than 13 years of teaching experience. He has received the University Gold Medal for securing the first position at B.Sc. and the University Silver medal for securing the second position at M.Sc. level. He received the Post-Graduate Merit Scholarship for University Rank Holder—awarded by the Ministry of HRD and UGC (2006–2008), Government of India. He also received the CSIR Junior Research Fellowship, which was awarded by CSIR-UGC (2008). He is actively engaged in research topics encompassing plant stress management by engineered nanoparticles and the synthesis of starch-based biofilms and edible coatings. He has published more than 40 articles with high-impact factors in reputed journals, such as *Water Research and Scientific Reports*, among many others. He has recently edited two books published by Springer and has also published several book chapters in edited volumes to date. He is a life member of prestigious societies like "The Indian Science Congress Association"; "Association of Food Scientists and Technologists (India)" and "Society of Plant Research (India)".

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Chapter 1
Fundamental Structure, Composition and Cutting-Edge Applications of Polysaccharides in the Contemporary Context

Mohan Das, Sayantan Santra, Moumita Chakraborty, Pritha Biswas, Subhara Dey, Ananya Pal, and Rintu Banerjee

Abstract With the advent of science and technology, study on polysaccharides has become convenient and easier to unravel several potential facts about the biomolecule. The basic architecture of polysaccharides derived from different sources presented distinctive unique features, which improved the understanding that natural polysaccharides not only bear storage properties but also play crucial role as structural element of many systems. The abundance of natural polysaccharides, its versatile nature makes it a saviour molecule for every domain of industries. This approach not only utilizes natural resources but also reduces the burden of wastes. The advent of modern processing technologies has successfully proven that the properties of these polymers can be enhanced and applied in different sectors like health care, food industry and energy sectors. Considering the importance of the situation researchers around the world, especially of European countries has been doing innovative and excellent piece of work in the aforesaid domain. Given the relevance of polysaccharides in the realm of therapeutic activities and biomedical applications, a generic reaction scheme of diverse polysaccharide molecular modifications is reflected. The molecular modification process, which involves chemical, physical,

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and biological approaches as well as the most important chemical processes, is thoroughly covered. The chapter aims to elaborate on the different natural sources of polysaccharides, basic chemistry and case studies based on the research performed by European researchers and scientists in the domain of polysaccharides.

Keywords Polysaccharides · Packaging · Skincare · Tissue engineering · Vaccination

Abbreviations

ALG	Alginates
CHT	Chitosan
CPS	Capsular polysaccharides
DGGE	Denaturing gradient gel electrophoresis
DMI	1, 3-Dimethyl-2-imidazolidinone
DNRS	Debranched normal rice starch
DP	Degree of polymerization
DSC	Differential scanning calorimetry
EC	European Commission
EPNOE	European polysaccharide network of excellence
EU	European Union
FTIR	Fourier transform infrared spectroscopy
GG-MA	Gellan gum methacrylated
GMA	Glycidyl methacrylate
HA	Hyaluronic acid
HMT	Heat moisture treatment
HPSEC/MALLS	High performance size exclusion chromatography with multi-angle laser light scattering
IVD	Intervertebral disc
LCA	Life cycle assessment
MAP	Modified atmosphere packaging
Mw	Molecular weight
NMR	Nuclear magnetic resonance
NP	Nucleus pulposus
NSP	Non-starch polysaccharides
PEM	Polyelectrolyte multilayers
PVA	Poly vinyl alcohol
RS	Resistant starch
SAXS	Small angle X-ray scattering
SCFAs	Short chain fatty acids
SDS	Slowly digestible starch
SEM	Scanning electron microscopy
SP	Starch-based polysaccharides

TEMPO	2,2,6,6-Tetramethyl, 1-piperidinyloxy
WAXD	Wide angle X-ray diffraction

1.1 Introduction

The evergreen meaningful communal debate on "Climate Change", housed from the United Nation Conference, organized in Copenhagen, December 2009, have evoked controversies round the genera of professions. Disparities in the opinion have tuned the voices of the leaders of politics, science and business people. Although, the explanations jointly raised a single agenda for transition towards a carbon– di– oxide (CO_2) neutral bio-based economy, being an utmost need for the sustainable future of our planet Earth. Since, the final outcome of CO_2 fixation is carbohydrate and the fundamental medium of exchange and message system among organisms, so the entire attention of the globe is on polysaccharides. The academic and industrial research members of European Polysaccharide Network of Excellence (EPNOE) were convinced to accept that polysaccharides are the central attraction point of tomorrow's research round the world in the field of food, energy, green chemical, medicine and health sector. This vision not only offers credible answers to the problems faced by researchers but also acts as a pathfinder to address the global issues and attain the goal of CO_2 neutral environment in sustainable manner. So, for the reason with the aid of European Commission (EC), EPNOE was established connecting 16 European Union (EU) research institutions and > 25 companies round the world, dealing with polysaccharide research and business. The 16 research institutions described have experts dealing with polysaccharide associated disciplines involving chemistry, biotechnology, microbiology, chemical engineering, mechanical engineering, material science, economics and life cycle assessment. Dated back in 2009, EPNOE described a road map for polysaccharide research and development that can aid as a guide for stakeholders involved in the business of carbohydrate polymers to identify areas and perform research for a purpose (Persin et al. 2011a, b). By nature, polysaccharides are repetitive structures of sugar moieties but by definition, they are natural macromolecular polymers composed of > 10 monosaccharide units bonded by glycosidic linkages having molecular mass of 10,000 to millions. On the basis of structure, classification of polysaccharides is convincing, unambiguous and satisfactory, with a few exceptions keeping aside. Naturally, polysaccharides are found in microbes, plants and animals. The existence and their respective function extremely vary widely among the kingdom and even among species of the same genera (Rasheed et al. 2020). Although native form of any polysaccharide possess unique in-built properties but sometimes cannot meet the demands for the applied purpose at a commercial scale, so specific controlled modification can fine tune the polymer and overcome such critical hurdles. In fact, sometimes extraction of the polysaccharide and its purity also raises troubles for its industrial-scale applications. The chapter mainly focuses in describing polysaccharides of different origin, their

unique characteristics and the progresses achieved by researchers of European continent, for their respective application in industrial scale (Persin et al. 2011a, b; Edgar and Navard 2015).

1.2 Origin, Localization and Structural Definition

1.2.1 Microbial Polysaccharides

1.2.1.1 Bacterial and Fungal Polysaccharides

The empirical observation historically reverses back to 1813, when sugar cane and sugar beet syrups were difficult to filter, since with time the liquid transforms into a quasi-solid to crystalline texture. In 1861, Louis Pasteur interpreted the observation is a result of microbial fermentation. Later in 1878, it has been identified that it was *Leuconostoc mesenteroides* and its respective product dextran is responsible behind such observation. As, sucrose stimulates expression of an enzyme capable of linking anhydro—glucose units by α (1 → 6) glycosidic linkages (Span and Marletta 2015). Actually, since the discovery, the transferase enzyme was used for dextran production in industrial scale and was regarded as the first microbial polysaccharide used as the basic component of stationary phase of chromatography columns. With the advent of progressive research, it is now well established that depending on the localization site, microbial polysaccharides can be classified into 3 groups, namely—(i) intracellular polysaccharides; (ii) cell wall polysaccharides; (iii) extracellular polysaccharides (Crescenzi 1995). The difference between polysaccharide content and location is depicted in the Fig. 1.1.

Peptidoglycan

Peptidoglycan, a structural component of almost all bacterial cell wall, is present outside the cytoplasmic membrane to preserve the turgor force and sustain the integrity of the cell (Fig. 1.1). It is inevitably associated with cell division and cell growth and so biosynthetic inhibition or enzymatic degradation of peptidoglycan may result in cell lysis. Scientific evidences reports that they are also present in 'Cyanelles' (photosynthetic organelle) of glaucocystophyte algae. Even as few as, nine and five biosynthetic genes were found in *Phycomitrella patens* (moss) and *Arabidopsis thaliana* (a green plant) respectively, which may impart roles in chloroplast division since no evidence of peptidoglycan were observed till date. Structurally peptidoglycan is made of glycan strands, composed of *N*-acetylglucosamine (Glc*N*Ac)and *N*-acetylmuramic acid (Mur*N*Ac) moieties bonded by β-(1 → 4) linkages, which are further crosslinked by short peptides composed of L-Ala-γ-D-Glu-meso-A_2pm (or L-Lys)-D-Ala-D-Ala (A_2pm, 2,6-diaminopimelic acid). The carboxyl

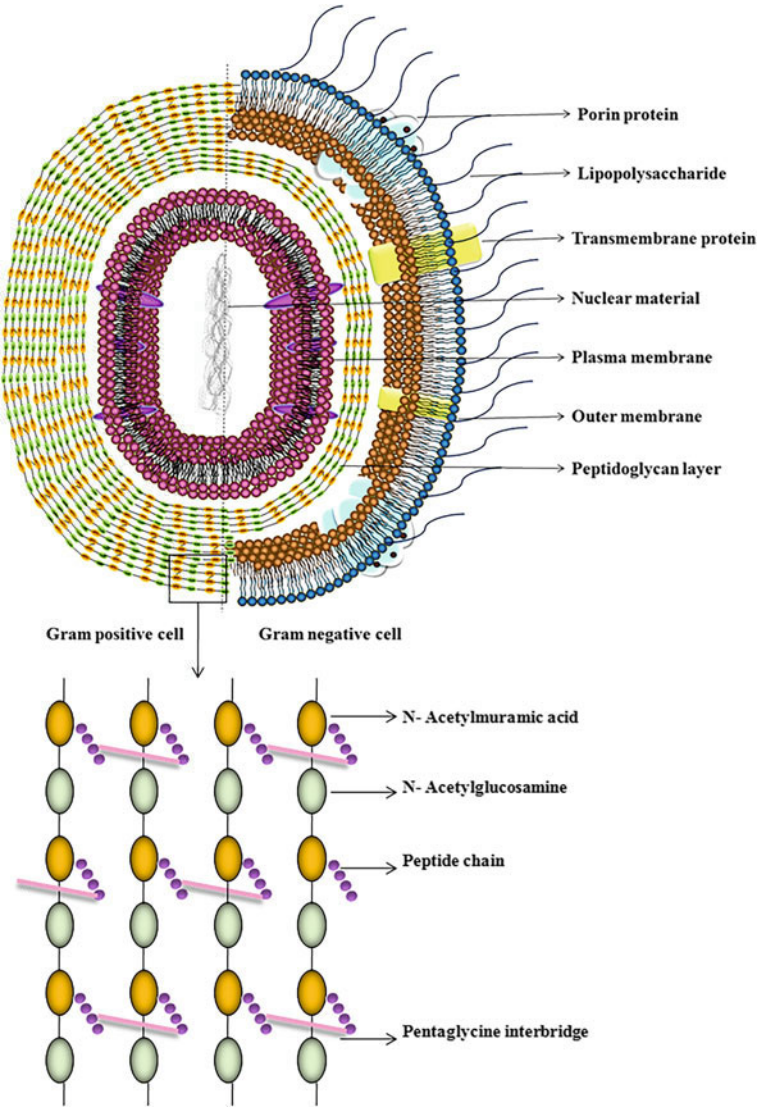

Fig. 1.1 Structural localization of polysaccharide and its compositional distinction between Gram (+) and Gram (−) bacterial cell

group of D-Ala at location 4 and the amino group of 2,6-diaminopimelic acid at location 3, aids in crosslinking the glycan strands (Egan et al. 2020; Vollmer et al. 2008).

Capsules

Capsules are extracellular bacterial polysaccharides, anchored intimately on the cell wall surface of both pathogenic gram positive (+) and gram negative (−) bacteria. They are covalently attached to the cell surface via Lipid A or phospholipid molecules but some are also attached with the cell even in absence of the membrane anchor. It has been reported that, capsular polysaccharides (CPS) is evidently found in species of *Escherichia, Pneumococci, Staphylococci, Streptococci, Meningococci, Pseudomonas, Klebsiella, Salmonella, Bacteroides, HaemophiIus* and *Clostridia.* They are clearly visible with the aid of light microscope after negative staining. Chemically, it is polymer of D-glutamic acid. Among all, the CPS of *Bacillus anthracis*, is well studied, since it is the negatively charged surface determinant and virulent part of the micro-organism (Ezzell and Welkos 1999; Kasper 1986).

Curdlan

Curdlan, a microbial polysaccharide, was first discovered by Harada et al. in 1966. During research activities with *Alcaligenes faecalis* var. myxogenes 10C3 strain, curdlan was discovered, although the investigation primarily aimed at succinoglycan production. Being an exopolysaccharide, curdlan is produced via three steps: (i) substrate processing, (ii) intracellular metabolism, (iii) excretion of the polymer from the cell. Curdlan is a linear homopolymer of D-glucose units, bonded by β-(1 → 3) glycosidic linkages, bearing an ~ molecular weight of $5.3 * 10^4$ and $2 * 10^6$ (Zhang and Edgar 2014).

Pullulan

Pullulan, is a water soluble, aerobically produced fungal exopolysaccharide. Its chemical structure is defined as a linear regularly repeating structural unit of maltotriose units (α, 1 → 4 linked, 3 glucopyranosyl moeities) bonded by α, 1 → 6 glycosidic bonds. Since, it is not a branched macromolecule; pullulan produced by polymorphic *Aureobasidium pullulans*, is the best studied and characterized glucan-based structure. The number-molecular weight (M_n) is ~ 100–200 kDa and weight—molecular weight (M_w) is ~ 362–480 kDa. The viscosity of aqueous solutions of pullulan is directly proportional to its molecular weight (Singh et al. 2008).

Dextran 40 and Dextran 70

Dextran, was discovered as the microbial by-product of wine production by Louis Pasteur. The structure varies extensively with the change of family, species, and strain. During the early 1950's, it was first introduced in the field of medicine and clinical practice by Swedish scientists. It is mainly a polymer of glucose linked by

α (1 → 6) linkage with distinctive branches by α (1 → 3) bond, which forms the characteristic feature to distinguish it from dextrin. Although, there are many forms, geometry, and molecular sizes of dextran but dextran 40 and dextran 70 with ~ molecular weight of 40,000 and 70,000, respectively, are under investigation; since their colloidal osmotic effect exerts distinctive volume expansion capacity for its use as a non-toxic plasma substitute (Díaz-Montes 2021; Atik 1967).

1.2.1.2 Algal Polysaccharides

Agar

Agar, a gelling hydrocolloid was accidentally discovered by Tarozaemon Minoya, an inn-keeper of Kyoto, Japan, during the mid of seventeenth century. In general, it is principally produced by *Gellidium amansii*. These species are mostly grown on rocky sea bottom. It has been reported that there are 55–59 species of European *Gellidium* and 24 species of Japanese *Gellidium*, producing agar but only 10 species were used for its commercial production. Agar is mainly composed of agarose and agaropectin, where the former is the main and simple constituent and the latter is a much complicated polysaccharide having uronic and sulphuric residues. Agarose is mainly composed of alternating 1, 3-linked β-D-galactopyranose and 1, 4-linked 3, 6-anhydro-α-L-galactopyranose residues and this disaccharide is also known as agarobiose. Although, the structure of agaropectin is not yet well understood but it has been reported that residues of sulphuric acid, pyruvic acid, D-glucuronic acid as well as minimal traces of D-xylose, 6-*0*-methylo-Galactose, L-galactose and 4-*0*-methyl-L-galactose were isolated from the polysaccharide (Matsuhashi 1990; Araki 1956).

Alginate

Alginates (ALG), is chemically defined as a group of anionic polysaccharides, naturally extracted from cell walls of *Macrocystis pyrifera*, *Ascophyllum nodosum and Laminaria hyperborean* (brown algae). The term alginate is mostly referred for alginic acid, its salts and its derivatives. It is a linear polysaccharide of α,L guluronic acid (G) and β,D mannuronic acid (M), bonded by 1 → 4 linkages and arranged in homologous (poly-G or M) or heterologous patterns (poly G and M). Inherent hydrophilicity, water absorption capacity, sol/gel property and viscosity of ALG are mostly defined by the presence of G-block and M-blocks. And for the reason, the molecular weight of ALG ranges from 33,000 to 400,000 g/mol. Increasing G-block content makes ALG gels stronger and brittle. ALG-crosslinking is mostly achieved by thermal gelation, "click-reaction", free radical polymerization, cell cross-linking, ionic cross linking and covalent cross linking. The G: M proportion can be achieved by enzymatic epimerization by functional catalyzation of mannuronan C-5 epimerasaes, isolated from *Azotobacter vinelandii*. The process

mostly enriches the guluronic acid content by converting mannuronic acid, without cleaving or effecting glycosidic linkages. Being a biodegradable polymer, it shares structural similarity with glycosaminoglycans and for the reason it possess active sites enabling better cellular attachment (Gheorghita Puscaselu et al. 2020; Szekalska et al. 2016).

Carrageenan

Carrageenan is considered as a generic name given to a family of polysaccharides, outsourced from species of *Rhodophyta* (red seaweeds). The extraction of these hydrophilic colloids initiated since 1810, in Ireland. Carrageenan is mainly the cell wall material of species belonging to the genera *Gigartina, Chondrus, Eucheuma and Hypnea.* Traditionally, there are six basic forms of carragenan and they are Theta (θ), Nu (ν), Mu (μ), Kappa (κ), Iota (ι) and Lambda (λ). Since, distinctive forms of carragenan are outsourced from different weed sources, so the aforementioned nomemclature is relevant both for commercial classification and chemical classification. A tropical seaweed named *Kappaphycus alvarezii*, is considered as the predominant producer of κ-carrageenan, whereas ι-carageenan is produced by *Eucheuma denticulatum.* The final outcome of the aforementioned forms is mostly achieved by transforming the biological precursor's μ and ν—carrageenan via alkali treatment at high temperatures (Guo et al. 2022). λ—carrageenan form is mostly extracted from *Chondrus spp.* and *Gigartina spp.* The sporophytic plants of the above mentioned seaweeds produce λ—carrageenan, whereas the gametophytic plants manufacture κ/ι-hydrid forms. Chemically, they are defined as linear hydrophilic sulphated galactans. They are mainly composed of 3-linked β-D-galactopyranose (G-units) and 4-linked 3, 6-anhydro-α-D-galactopyranose (DA-units) or α-D-galactopyranose (D-units), which forms a disaccharide and acts as repeating units. The position and number of sulphated groups and the existence of 3, 6-anhydro-bridge over 4-linked-galactose residue, forms the basis of classification of sulphated glycans. In general cases, the molecular weight of commerecial carrageenan mostly ranges from 100 to 1000 kDa (Campo et al. 2009).

1.2.2 Plant-Based Polysaccharides

Plant polysaccharides are broadly categorized into two distinct chemically well defined types: (i) starch based polysaccharide (storage energy form) and (ii) non-starch based polysaccharides (structural form) as depicted in Fig. 1.2. Starch-based polysaccharides (SP) being composed of amylose and amylopectin are easily digestible by pancreatic enzymes whereas non-starch polysaccharides (NSP) are resistant to pancreatic enzymes and are only utilized by gut microbiota via fermentation. Polysaccharides involved in the architectural arrangement of cell walls are specific to the types of cell. Cellulose, hemicelluloses (arabinoxylans, mannans,

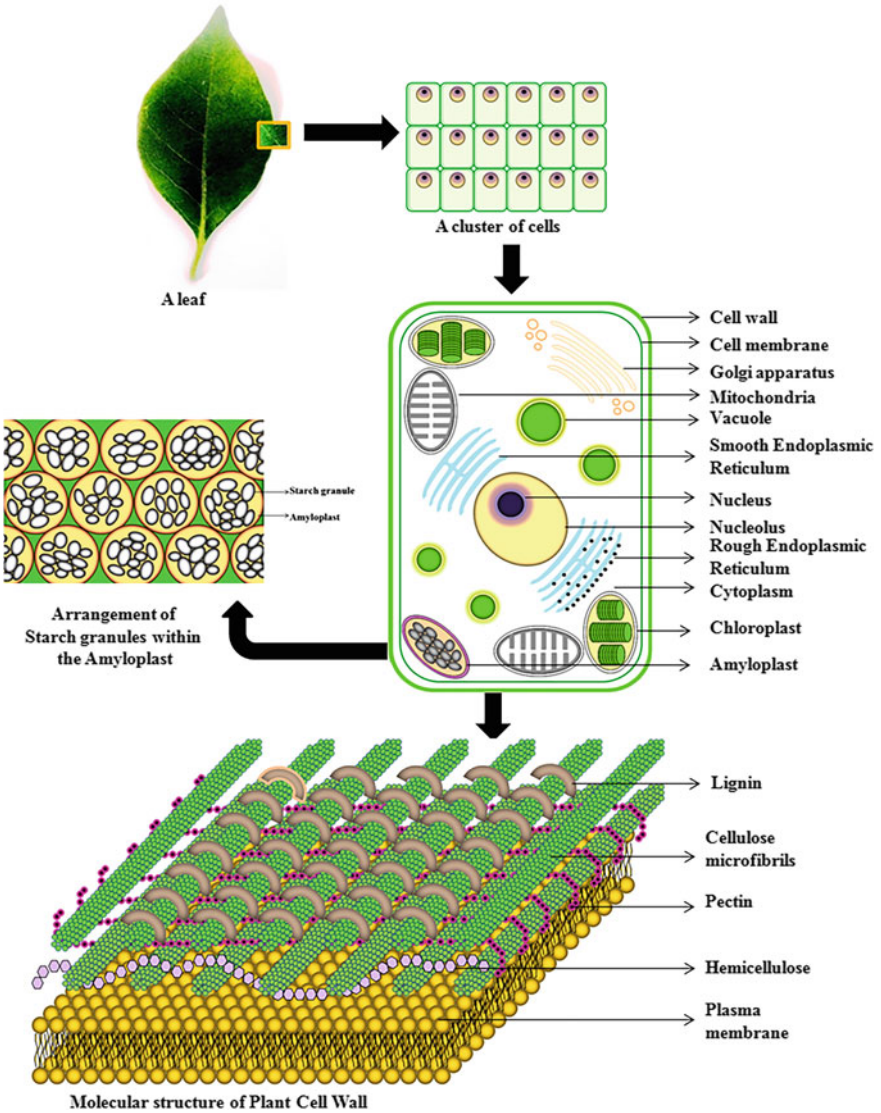

Fig. 1.2 Diagrammatic localization of structural and storage forms of plant polysaccharides

glucomannans, galactoglucomannans, xylans, glucoxylans) and pectic polysaccharides (arabinans, arabinogalactans, galactans, galacturonans, rhamnogalacturonans) are considered as NSPs, playing distinctive role in different structural arrangement of plant cells (Englyst 1989; Das et al. 2022a).

1.2.2.1 Starch-Based Polysaccharides

Starch granules are synthesized and localized within amyloplasts as discrete molecules in a broad spectrum of tissues of different plant species. The morphology of starch granules is very dynamic, since its shape may be round, lenticular, oval, or polygonal; size distribution may range from 2 to 100 μm in diameter; arrangement may be simple or clustered. In lieu to the diversity in morphology, it is astounding that the internal architecture is universal regardless of the plant organ. It is believed that the hilum is the origin point from where the entire granule grows. And for this reason, when observed under rays of cross-polarized light of an optical microscope a "Maltese cross" is observed, which is a typical feature of all starch granules (Das et al. 2023). There are two types of α-glucan molecules, namely amylose and amylopectin, which approximately fill 98–99% dry weight of starch granules. However, the ratio of amylose and amylopectin varies in accordance with its botanical origin (Das et al. 2022a; Das and Banerjee 2022). The structural composition of amylose is elaborated as a linear chain of α-glucan units linked around 99% by α (1 → 4) glycosidic bond. It possesses a molecular weight of ~ $1 * 10^5$–$1 * 10^6$ with a degree of polymerization (DP) of 324–4920 having around 9–20 branch points bonded by α (1 → 6) glycosidic linkages, which although greatly differs and depends on the botanical origin of the granule. In comparison to amylose, amylopectin is a bulky molecule having a molecular weight of ~ $1 * 10^7$–$1 * 10^9$ and a DP of 9600–15,900. The structural architecture of amylopectin involves a heavily branched structure with obvious branching of around 5% by α (1 → 6) glycosidic bonds and the linear form by rest 95% of α (1 → 4) glycosidic bonds. However, the unit chains of amylopectin are relatively shorter in comparison to amylose. In native form, the branches of amylopectin form double helices, which in turn represent the crystalline lamellar structure, showing different X-ray diffraction patterns. As a result, it is the amylose—amylopectin ratio which defines the allomorph of any starch granule as A, B, or C type (Tester et al. 2004; Bertoft 2017; Das et al. 2022b).

1.2.2.2 Non-starch Based Polysaccharides

Cellulose

Cellulose, being the most abundant polysaccharide on planet Earth is a linear homopolymeric chain of D-anhydroglucose, bonded by β, D-glycosidic linkages. Although nowadays, it is outsourced from microbial sources but cellulose is the main structural component of cell wall of plants. In 1839, a French Chemist, Anselme Payne first isolated cellulose from plant material. In general, the degree of polymerization (DP) of natural cellulose is 10,000, but in the case of cotton, it is 15,000. Cellulose, in general, has four polymorphs, cellulose I, II, III, and IV. Cellulose I is the most crystalline type natural polymorph existing in two forms: I_α and II_β, which by chemical nature are similar to each other but differ in the packaging pattern within the lattice. Recrystallization of cellulose I causes the rearrangement of

parallel chains, promoting the formation of anti-parallel strands, which move down to generate cellulose II. Mercerization and regeneration are two of the suggested routes by which cellulose II can be formed. Cellulose III_1, or cellulose III_2, is mostly prepared by heating cellulose I or cellulose II in liquid ammonia. Upon heating the corresponding forms in glycerol, cellulose IV_1 or cellulose IV_2 gets produced. In plants, cellulose forms a composite structure with hemicelluloses and lignin, which makes its extraction difficult. However according to research results, cellulose can be successfully isolated from wood, flax, hemp, cotton, and sisal. Cryo-crushing, enzymatic pre-treatment, TEMPO-mediated oxidation are a few of the effective technologies adapted for cellulose extraction to reach the possible purity level (Suhas Gupta et al. 2016).

Pectic Polysaccharides

Pectic polysaccharides, a structural component of dicotyledonous plant cells walls is considered to be the most complex polymer, described after cellulose and hemicelluloses. It is deposited within the middle lamella, primary and secondary cell walls during the early stages of cell expansion. Pectin belongs to the family of plant cell wall polysaccharides rich in galacturonic acid covering homogalacturonan, xylogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II (Santra et al. 2023). The complexity of the molecule can be estimated by observing the array of 67 transferases (mostly glycosyl transfersases, methyl transferases and acetyltransferases) playing crucial roles in the pectin biosynthetic pathway. It has been observed that pectin is mostly composed of 70% of galacturonic acid, linked at *O*-1 and *O*-4 positions, where a quantifiable proportion of acid residues are present as methoxyl esters and a few in neutral forms as side chains. According to De Vries, there exists a "smooth" pattern and a "hairy" ramified region, which is mostly, composed of neutral sugar moieties (Mohnen 2008; Voragen et al. 2009).

Gum Arabic

The oldest and best known natural gum ever found in the history is Gum Arabic or Gum Acacia. It is tree exudates of *Acacia senegal* and *Acacia seyal*. During third millennium B.C., ancient Egyptians used them as an article of commerce. It is a branched polysaccharide, where the main backbone and the side chain are composed mainly of 1, 3-linked β-D-galactopyranosyl units. The side chain contains only two-five β-D-galactopyranosyl units, linked to the main backbone by 1 → 6 linkages. The presence of α- L-rhamnopyranosyl, α-L-arabinofuranosyl, β-D-glucuronopyranosyl and 4-*O*-methyl-β-D-glucuronopyranosyl are universally found in both main and side chains, but the later are mostly present as end units. The gum exudates from *Acacia seyal* have low α-L-rhamnopyranosyl and β-D-glucuronopyranosyl units as compared to *Acacia senegal* exudates (Verbeken et al. 2003).

Gum Karaya

The name "Gum Karaya" was given to the dried exudate of *Sterculia urens* tree. To obtain the gum exudate, 4-inch-deep holes were made on the tree; so that the sap oozes out of the tree and form lumps of 5 pounds in size. The chemistry of gum karaya has been a topic of concern because of its acidic nature. Naturally, the gum is partially acetylated and its acid number varies from 13.4 to 22.7. It is mainly composed of a backbone of α-D-galacturonic acid and α-L-rhamnose moieties. The branched side chain is either (1 → 2) linked β-Dgalactose or (1 → 3) linked β-D-glucuronic acid, attached to the galacturonic acid unit of the main backbone, whereas (1 → 4) linked β-D-galactose moieties are found in case of rhamnose residues. Due to its complex architecture, the gum exudate bears a molecular weight of 16 * 10^6 Da (Verbeken et al. 2003; Goldstein 1954).

Gum Tragacanth

Gum Tragacanth, is a heterogenous, highly branched, complex, natural polysaccharide, mostly obtained from *Astragalus spp.* in the form of potassium, magnesium and calcium salts. The gum exudate is mainly composed of two fractions, (i) Bassorin or tragacanthic acid and (ii) Tragacanthin. The former component is insoluble in water but bears the ability to swell, whereas the later is water soluble. The main chain of tragacanthic acid is mainly composed of α (1 → 4) linked D-galactose residues, with (1 → 3) linked branched residues of D-xylose. The swelled form of tragacanthic acid takes a rod-like molecular shape, whereas water soluble tragacanthin possess a spherical shape. The core structure of, tragacanthin mainly composed of (1 → 3) and (1 → 6) linked D-galactose residues, having branches of L-arabinose linked by (1 → 2), (1 → 3) and (1 → 5) glycosidic bonds. The molecular weight of tragacanth is ~ 8.4 * 10^5 Da (Verbeken et al. 2003).

1.2.3 Animal Polysaccharides

Polysaccharides are ubiquitously present in animals, mostly in crustaceans, insects and mammals. Similar, to other systems they are also present either as structural component or storage material (Fig. 1.3). Some of the important polysaccharides outsourced from animals are described in the following section.

1.2.3.1 Hyaluronan

In 1934, Karl Meyer and John Palmer discovered hyaluronan from the vitreous of bovine eyes. It is a naturally occurring mucopolysaccharide, widely found in connective tissues, umbilical cords, vitreous of eyes, in concentrated synovial fluid

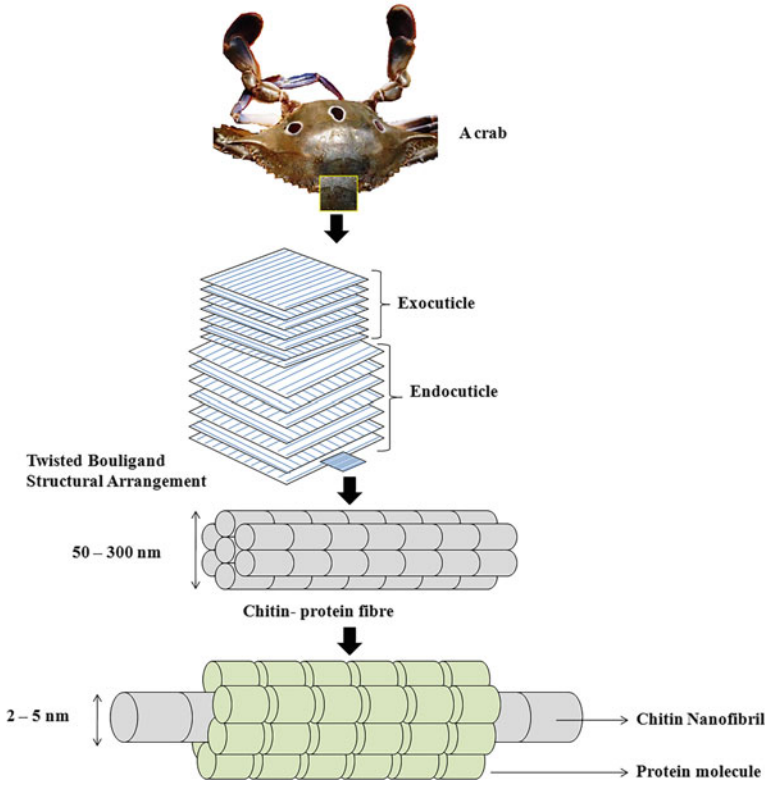

Fig. 1.3 Structural localization of polysaccharides in crustaceans

and chicken combs. This extracellular matrix, is mainly composed of a repeating dissacharide containing D-glucuronic acid and D-N-acetyl glucosamine moieties bonded by alternating β (1 → 4) and β (1 → 3) glycosidic bonds. The number of disaccharide repeats can reach up to 10,000 or more and a molecular weight of ~ 4 million Da. Hyaluronan biosynthesis is a highly controlled procedure, where a group of hyaluronan synthases, with three types in vertebrates, HAS1, HAS2 and HAS3, plays the crucial role in the biosynthetic pathway (Necas et al. 2008).

1.2.3.2 Chitin

Chitin is considered as the second abundant polysaccharide after cellulose. Naturally, it was first identified in 1884 from the exoskeletons of arthropods. It is further categorised into α and β forms. α-Chitin is abundantly present in krill, in crab tendons, in lobster, cone snails harpoons and spine of *Sagitta*. The unique structural properties of α-chitin presents a highly crystalline ordered structure along high percentage purity since it is produced in absence of protein, pigment or calcite. B-chitin is the

rarer form and mostly present in association with proteins of squid pens and tubes of vestimetiferan and pogonophoran worms. Chitin is composed of repeating units of *N*-acetyl-D-glucosamine, linked by β (1 → 4) glycosidic bonds. Single crystal of pure chitin is mostly of low molecular weight (Rinaudo 2006).

1.2.3.3 Glycogen

Glycogen is the storage form of polysaccharide, mostly present in liver and muscle tissue of mammals, molluscs, fish, insects and in many other animals. The architecture of glycogen resembles to a tiered, bush like structure, bearing a backbone of glucopyranosyl units bonded by α (1 → 4) linkages having branches of same units attached by α (1 → 6) glycosidic linkages. Because, of its ability to provide energy to liver and muscle it is always present in dynamic state. The molecular weight of glycogen varies from $1 * 10^6$ to $2 * 10^9$, which mostly depends on the type of tissue, nutritional state of the host, time of the day, temperature and also on the immunogenic status (Bemiller 2008).

1.3 Basic Chemistry and Mechanisms of in Vivo and in Vitro Modifications

Polysaccharides are the condensed form of polymers where the building blocks are the monosaccharides bonded together by *O*-glycosidic linkages. The *O*-glycosidic linkages between monosaccharides are formed due to condensation or dehydration reaction. Polysaccharides are classified as linear or branched, based on the existence of the array of stereo and region-type glycosidic bond connecting the sugar moieties, like α-(1 → 2)-, α-(1 → 3)-, α-(1 → 4)-, α-(1 → 6)-, β-(1 → 2)-, β-(1 → 3)-, β-(1 → 4)-, and β-(1 → 6)-linkage (Xiao and Grinstaff 2017; Kadokawa 2016; Navarro et al. 2019; Yalpani 1988).

1.3.1 Synthesis, Substitutions and Derivatives

1.3.1.1 Chemical Method

Polysaccharides plays very crucial role in biological processes and thus majority of research is based upon interpreting the role of polysaccharide in living system and explain the mechanism at the chemical level. The major problem of polysaccharide research is the lack of abundant and unfailing sources, its mode of extraction, purification and batch-to-batch variation in molecular weights, composition and branching. Thus, the chemical synthesis of polysaccharide enables to study the